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Comparing pretreatment methods for improving microalgae anaerobic digestion: thermal, hydrothermal, microwave and ultrasound

Fabiana Passos[1], Javier Carretero[1] and Ivet Ferrer[1*]

[1] GEMMA – Environmental Engineering and Microbiology Research Group, Department of Hydraulic, Maritime and Environmental Engineering, Universitat Politècnica de Catalunya·BarcelonaTech, c/ Jordi Girona 1-3, Building D1, E-08034, Barcelona, Spain

[*] Corresponding author:

Tel.: +34 934016463

E-mail address: ivet.ferrer@upc.edu

Abstract

The anaerobic digestion of microalgae is hindered by its complex cell wall structure and composition. Thus, several pretreatment methods have been used for increasing microalgae anaerobic biodegradability. Since the methane yield depends on biomass characteristics, pretreatments should be compared using the same microalgal biomass. In this study, physical pretreatments including thermal (95 ºC; 10 h), hydrothermal (130 ºC; 15 min), microwave irradiation (900 W; 3 min; 34.3 MJ/kg TS) and ultrasonication (70 W; 30 min; 26.7 MJ/kg TS) were evaluated in terms of microalgae solubilisation and methane yield increase in batch tests. Organic matter solubilisation was improved in all cases, with the highest increase on soluble proteins, followed by soluble carbohydrates and soluble lipids. This was attributed to the macromolecular and cell wall composition of the main microalgae species composing the biomass, i.e. *Monoraphidium* sp. and *Stigeoclonium* sp. Furthermore, the methane yield was increased by 72% for thermal, 28% for hydrothermal and 21% for microwave pretreatments, whereas no significant increase was found for ultrasonication as compared to control. Outstanding results of the thermal pretreatment should be validated in prospective pilot-scale studies in order to quantify the potential increase in biogas production upon continuous operation.

Keywords

Algae; Anaerobic digestion; Bioenergy; Biogas; Methane; Solubilisation

1. Introduction

In the last decades microalgae production and processing for bioenergy purposes has been a trending topic of research. Most published literature in the field is focused on biodiesel and biogas generation. However, biodiesel production has high energy and economic impacts for drying the biomass and extracting the lipid content of microalgae cells. In fact, it has been shown that viable microalgae biofuel production in full-scale systems is only possible if all processes are optimised and integrated in a biorefinery approach (Rawat et al., 2013). Particularly, anaerobic digestion has been considered a crucial step for recovering energy from residual biomass after lipid extraction (Ramon-Suárez and Carreras, 2014). Anaerobic digestion is a consolidated technology, which may be also used for converting the whole microalgal biomass into biogas, without previous drying and extracting steps.

The drawback of microalgae anaerobic digestion relies on its complex cell wall structure and composition, which hampers the hydrolysis step. In this context, pretreatment methods have been applied for improving the methane yield and/or conversion rate of microalgae and other complex organic substrates (Cesaro and Belgiorno, 2014; Passos et al., 2014a). Studies comparing the effect of different pretreatments on microalgae showed how intensive techniques involving high temperatures and pressures (170 ºC and 6 bars) or high specific energies (100-130 MJ/kg TS) reached the highest methane yield increase (Alzate et al., 2012; González-Fernández et al., 2012), but they also require a high energy input.

In our previous studies thermal, hydrothermal, microwave and ultrasound pretreatments were effective at increasing both biomass solubilisation and methane yield (Passos et al., 2013a; 2013b; Passos et al., 2014b; Passos and Ferrer, 2015). For each method, biochemical methane potential (BMP) tests were carried out under different pretreatment conditions in order to select the best ones based on experimental results (Table 1). However, from these studies it is not possible to elucidate which is the best pretreatment technique, since microalgal biomass was not the same in all of them. Indeed, when microalgal biomass is grown in high rate algal ponds (HRAP) treating wastewater, a spontaneous

mixed culture of microalgae and bacteria is produced. This biomass varies over time due to many factors, such as environmental conditions (e.g. solar radiation, temperature and precipitation), wastewater composition (e.g. presence of bacteria and toxic compounds) and occurrence of microfauna (e.g. rotifers) (Park et al., 2011). Species variation together with the fact that microalgae biodegradability depends on the characteristics of the cell structure and composition, calls for pretreatment methods comparison using the same microalgal biomass.

Therefore, the aim of this study was to compare different mechanical and thermal pretreatments in terms of biomass solubilisation and methane yield increase in BMP tests using the same microalgal biomass. Thermal, hydrothermal, microwave and ultrasound pretreatments were applied under the best conditions found in previous experiments (Table 1). Biomass solubilisation was evaluated in terms of total organic matter solubilisation (i.e. volatile solids) and soluble proteins, carbohydrates and lipids (i.e. fatty acid methyl esters (FAME)) concentration. BMP tests were used for evaluating the digestion rate and methane yield improvement after each pretreatment.

2. Materials and Methods

2.1 Microalgal biomass

Microalgal biomass consisted of a mixed culture of microalgae and bacteria mainly composed by green microalgae (*Stigeoclonium* sp. and *Monoraphidium* sp.) and diatoms (*Nitzschia* sp. and *Navicula* sp.) The biomass was grown in a pilot HRAP used for urban wastewater treatment. The experimental set-up was located outdoors at the laboratory of the GEMMA research group (Universitat Politècnica de Catalunya) in Barcelona (Spain). The HRAP received the primary effluent from a settling tank which had a useful volume of 7 L and a HRT of 0.9 hours. The primary effluent was pumped to the HRAP, which consisted of a PVC raceway pond with a paddle-wheel for mixed liquor stirring. The HRAP had a useful volume of 470 L and was operated with a HRT of 8 days. Microalgal biomass was harvested from secondary settlers with a useful volume of 9 L and a HRT of 9 hours. Following, biomass was

thickened in laboratory gravity-settling cones at 4 ºC for 24 hours for reaching total solid (TS) concentration of 3.0 % (w/w). Average characteristics of harvested biomass are summarised in Table 2.

2.2 Pretreatment methods

Four physical pretreatment methods were evaluated: thermal, hydrothermal, microwave irradiation and ultrasonication. Pretreatment conditions were selected according to our previous studies comparing different pretreatment conditions in BMP tests (Passos et al., 2013a; 2013b; Passos et al., 2014b; Passos and Ferrer, 2015) (Table 1). All pretreatments were carried out in glass bottles of 250 mL containing 150 mL of microalgal biomass. On the whole, 2 L of the same harvested microalgal were used, which allows for comparison between pretreatment methods.

The thermal pretreatment was carried out in an incubator under continuous stirring at 95 ºC for 10 hours, and the hydrothermal pretreatment was performed in an autoclave at 130 ºC and 1.7 bars for 15 minutes. Bottle caps slightly loose. After reaching the target temperature, biomass was maintained under this condition during the whole exposure time and afterwards pressure was gradually released to reach atmospheric conditions.

The microwave pretreatment was carried out in a household type microwave (Samsung M1914, 2450 MHz frequency) with an output power of 900 W and an exposure time of 3 minutes. The applied specific energy (34.3 MJ/kg TS) was calculated according to Eq. 1.

$$Specific\ energy\ (MJ/kg\ TS) = \frac{Power\ (W) x\ Time\ (s)}{Sample\ weight\ (kg\ TS) x\ 100} \quad \text{(Eq. 1)}$$

Finally, the ultrasound pretreatment was evaluated using a HD2070 Sonopuls Bandelin Ultrasonic Homogenizer device, equipped with a MS 73 titanium microtip probe, working with an operating frequency of 20 kHz. Ultrasonication was performed with an output power of 70 W and an exposure time of 30 minutes. As for microwave pretreatment, the applied specific energy (26.7 MJ/kg TS) was calculated according to Eq. 1.

2.3 Organic matter solubilisation

The soluble organic matter content in pretreated and non-pretreated microalgal biomass was compared. On the one hand, the soluble volatile solids (VS_s) concentration was measured for evaluating the total organic matter solubilisation. On the other hand, proteins, carbohydrates and lipids solubilisation was analysed using as indicators the increase in soluble proteins (ON_s), carbohydrates (glu_s) and fatty acid methyl esters ($FAME_s$), respectively.

The solubilisation increase for total organic matter, carbohydrates, proteins and lipids was calculated according to the following equations (Eq. 2-5), where sub-indexes refer to pretreated (p) and non-pretreated (o) biomass.

$$S_T\ (\%) = \frac{[(VS_s)_p - (VS_s)_o]}{(VS_s)_o}\ 100 \quad \text{(Eq. 2)}$$

$$S_C\ (\%) = \frac{[(glu_s)_p - (glu_s)_o]}{(glu_s)_o}\ 100 \quad \text{(Eq. 3)}$$

$$S_P\ (\%) = \frac{[(ON_s)_p - (ON_s)_o]}{(ON_s)_o}\ 100 \quad \text{(Eq. 4)}$$

$$S_L\ (\%) = \frac{[(FAME_s)_p - (FAME_s)_o]}{(FAME_s)_o}\ 100 \quad \text{(Eq. 5)}$$

2.4 Biochemical methane potential test

The anaerobic digestion rate and extent of pretreated and non-pretreated microalgal biomass were assessed by means of BMP tests. Digestate from a full-scale anaerobic reactor treating sewage sludge in a wastewater treatment plant near Barcelona (Spain) was used as inoculum (Table 2). BMP bottles had a total volume of 160 mL and a useful volume of 100 mL. Each bottle contained 18 g of microalgal

biomass (0.57 g TS) and 44 g of inoculum (1.46 g TS), corresponding to a substrate/inoculum ratio of 0.5 g COD/g VS (Passos et al., 2013a), and was filled with deionised water until 100 mL. pH values of substrate and inoculum were measured (Table 2). Since none of the applied pretreatment methods modified the pH, it was not corrected for BMP tests. The bottles were flushed with Helium gas (He), sealed with butyl rubber stoppers and incubated at 35 ºC until biogas production ceased. A blank treatment was used to quantify the amount of methane produced from the inoculum. Each BMP was performed in triplicate.

Biogas production was determined periodically by measuring the pressure increase with an electronic manometer (Greisinger GMH 3151). After each measurement gas was released until atmospheric pressure was reached. Samples from the headspace volume were taken every 2-3 days, to determine biogas composition (CH_4/CO_2) by gas chromatography (GC).

Accumulated volumetric methane production (mL) was calculated from the pressure increase and methane content in biogas, expressed under standard conditions. Methane yield was calculated by dividing the accumulated volume of methane produced by the VS content in each bottle (mL CH_4/g VS). The net value of methane yield was obtained by subtracting the production of the blank bottle.

2.5 Analytical methods

All physical-chemical analyses were carried out in triplicate and results are given as mean values. Microalgal biomass and sewage sludge were characterised by the concentration of TS, VS and chemical oxygen demand (COD), according to standard methods (APHA, AWWA, WPCF, 1999). pH was analysed with a Crison Portable 506 pH-meter. Microalgal biomass macromolecular composition was expressed as percentage of proteins, carbohydrates and lipids over the volatile solid (VS) content. Carbohydrate content was determined by phenol-sulphuric acid method after acid hydrolysis and measured by spectrophotometry (Spectronic Genesys 8). Protein content was determined from the total Kjeldahl nitrogen (TKN), which was analysed according to standard methods (APHA, AWWA, WPCF,

1999), using a TKN/protein conversion factor of 5.95 (López et al., 2010). Lipid content was determined by the Soxhlet extraction method (APHA, AWWA; WPCF, 1999).

Soluble fractions of volatile solids, proteins and carbohydrates were analysed from filtrated microalgal biomass samples. Soluble samples were obtained by centrifugation (UNICEN20, 4200 rpm, 8 min, 20 ºC) and filtration (glass fiber filter 47 mm and pore size 1 μm). Soluble FAME were used as indicator for soluble lipids and were analysed as follows. Firstly, extraction was carried out with a ratio of 8:4:3 (v/v) of chloroform:methanol:sample, i.e. 27.6 mL of chloroform, 13.3 mL of methanol and 10 mL of soluble biomass sample. The organic phase was collected after centrifugation (2000 rpm; 5 min) and 8 mL of KCl were added. Nitrogen steam was used for evaporation at 40 ºC. Following, for saponification, 0.4 mL of NaOH 0.5 N (as methanol) were added and the sample was heated at 100 ºC for 5 min. Afterwards, methylation was carried out by adding 0.4 mL of BF_3/Methanol (10% v/v; Supelco 3-3356) and heating at 100 ºC for 5 min. A known volume of hexane and 8.5 mL of saline solution (NaCl/water) was added to quench the reaction. The recovered organic phase was then pooled and spiked. The sample was placed in a chromatography vial of 2 mL with 1 mL of sodium sulphate anhydride. Nitrogen evaporation was carried out, followed by replacement with hexane. FAME content was measured using a GC, equipped with a capillary column (DB-23 Agilent, 30 m x 0.25 mm ID x 0.25 Micron film) and a flame ionization detector (FID). Helium was used as carrier gas, with a split ratio of 25:1 (column flow 1 mL/min). The system was calibrated with FAME reference standard mix containing 10 mg/mL (Supelco 37, catalog number 47885-U).

The methane content in biogas was measured by GC with a Thermal Conductivity Detector as described previously (Passos et al., 2013b).

Microalgae species identification was carried out using optic microscopic images (Aixoplan Zeiss, Germany), equipped with a camara MRc5, using the software Axioplan LE. Basic microalgae diversity morphotypes were identified from classical specific literature (Palmer, 1962; Bourelly, 1966).

2.6 Statistics

The statistical significance of experimental results was evaluated by the ANOVA and Tukey tests, with a significance level (α) of 5%, using R Commander Statistical Software. Anaerobic digestion in BMP tests was modelled by 1st order kinetics, fit by the least square method.

3. Results and Discussion

3.1 Microalgal biomass solubilisation after pretreatment

Microalgal biomass solubilisation after thermal and mechanical pretreatments is shown in Table 3. As can be seen, total volatile solids solubilisation was enhanced after all pretreatments as compared to non-pretreated microalgal biomass. The highest VS solubilisation increase was attained for the thermal pretreatment (20-fold) followed by hydrothermal pretreatment (9-fold), while it was similar for microwave (8-fold) and ultrasound (7-fold) pretreatments.

In order to identify the main organic macromolecules (i.e. proteins, carbohydrates and lipids) solubilised after each pretreatment, the content of soluble proteins, carbohydrates and FAME was analysed before and after pretreatments. FAME were used as indicator of lipids solubilisation. Pretreated microalgal biomass had higher content of all soluble organic macromolecules than non-pretreated biomass (Table 3). For thermal pretreatment, the highest increase was observed for soluble proteins (51-fold), followed by soluble carbohydrates (30-fold) and soluble lipids (13-fold). A similar trend was found for microwave and ultrasound pretreatments, where the highest increase was also attained for soluble proteins (23-fold increase for microwave and 18-fold increase for ultrasound pretreatments), followed by soluble carbohydrates (12-fold for microwave and 9-fold increase for ultrasound pretreatments), while soluble lipids reached the lowest increase (2-fold and 3-fold for microwave and ultrasound pretreatments, respectively). A different trend was observed for hydrothermal pretreatment, where the highest increase was attained for soluble lipids (31-fold),

followed by soluble proteins (12-fold) and soluble carbohydrates (11-fold), when compared to control. In fact, the thermal pretreatment in the case of soluble proteins and carbohydrates, and the hydrothermal pretreatment in the case of soluble lipids reached significantly higher values than mechanical techniques and non-pretreated biomass. The results suggest a positive effect of high temperature (> 100ºC) on FAME solubilisation, which was not observed at low temperature (< 100ºC). Conversely, low temperature pretreatment (<100ºC) for a longer exposure time (10 h vs. 15 min) had a positive effect on proteins and carbohydrates solubilisation. Indeed, the exposure time was considerably higher for the thermal pretreatment (10 h) than for the rest (1-30 min).

On the whole, proteins (12 to 51-fold increase) and carbohydrates (9 to 30-fold increase) accounted for the highest organic matter solubilisation, since soluble lipids were only increased from 2 to 13-fold, in respect to control. Such high solubilisation of proteins may be explained by the macromolecular composition of microalgal biomass, with 58% proteins over the total VS content (Table 1). Regarding the composition of microalgae cell wall, which is the most external layer of the microorganism, green microalgae are composed of polysaccharides (such as cellulose and hemicellulose) and glycoproteins (González-Fernández et al., 2011; Passos et al., 2014a). In this study, microalgal biomass was mainly composed by the species *Stigeoclonium* sp., *Monoraphidium* sp. and the diatoms *Nitzschia* sp. and *Navicula* sp. The filamentous microalgae *Stigeoclonium* sp. is a common species in wastewater treatment systems, tolerant to a wide range of water conditions and with ability to grow in waters polluted by organic matter and/or heavy metals (Kim et al., 2014). Its cell wall is mainly composed by cellulose and pectic substances (Dawes, 1966). The cell wall of *Monoraphidium* sp. is composed by 47% of neutral sugars, 16% of proteins, 6.1% of uronic acids, 0.4% of glucosamine and 31% of unknown compounds, on a dry weight basis (Blumreisinger et al., 1983). Differently from the former species, diatoms are composed by nanopatterned silica (SiO_2) and partly of pectic substances (Sumper and Brunner, 2006). According to this, the high carbohydrate and protein solubilisation after pretreatments may have been originated from microalgae cell wall compounds.

Monoraphidium sp. and *Stigeoclonium* sp. were possibly the main species affected by the pretreatment step, since diatoms have a resistant cell wall which is hardly biodegraded even after pretreatment (Passos and Ferrer, 2014).

In our previous studies, microscopic images were used to understand the pretreatment effect on microalgae cell structure. After thermal, hydrothermal and microwave pretreatments, most microalgae cells were damaged beyond repair, with unrecognizable intracellular organelles. For microwave pretreatment, images of pretreated *Monoraphidium* sp. showed how, although the cell wall was not completely cleaved, it was impaired, which may have increased organic molecules bioavailability (Passos et al., 2014c). Moreover, microscopic images of microalgae before and after anaerobic digestion were analysed for thermally pretreated and control biomass. In this case, results showed how pretreatment effectiveness and anaerobic biodegradability were species-specific. Indeed, species with less complex cell structure were solubilised after pretreatment (e.g. *Monoraphidium* sp.), while more complex species (e.g. *Stigeoclonium* sp. and *Scenedesmus* sp.) were damaged after pretreatment and mostly degraded in the anaerobic reactor. Finally, complex cells with resistant cell wall (e.g. diatoms) were not biodegraded even after thermal pretreatment (Passos and Ferrer, 2014). For hydrothermal pretreatment, images of *Oocystis* sp. cells showed how the external layer of the cell wall was cleaved and most pretreated cells biodegraded (Passos and Ferrer, 2015).

To sum up, thermal pretreatment (95 ºC, 10 h) reached the highest solubilisation, mainly due to the increase in soluble proteins and carbohydrates. This increase was most likely caused by the high content of proteins in microalgal biomass and by cellulose, hemicellulose and glycoproteins composing microalgae cell wall.

3.2 Effect of pretreatments on the anaerobic digestion rate and extent

Results from BMP tests are shown in Figure 1. The anaerobic digestion rate was 0.11 d^{-1} for non-pretreated and 0.11-0.13 d^{-1} for pretreated microalgal biomass, so none of the studied pretreatments

enhanced the reaction rate (Table 4). In respect to the methane yield, it was statistically higher for thermal (181 mL CH_4/g VS), hydrothermal (135 mL CH_4/g VS) and microwave (128 mL CH_4/g VS) pretreatments as compared to control (106 mL CH_4/g VS). Thermal pretreatment was the most effective, with 72% increase in methane yield compared to non-pretreated biomass. Hydrothermal and microwave pretreatments increased the methane yield by 28 and 21%, respectively, while ultrasound pretreatment (8% increase) was not significantly higher than non-pretreated microalgae (Table 4).

As expected from biomass solubilisation results, the thermal pretreatment attained the highest methane yield. This was attributed to the increased concentration of soluble organic compounds, in particular to soluble proteins and carbohydrates. Such a high solubilisation seemed to enhance the anaerobic digestion extent rather than the digestion rate. In fact, a positive correlation was found between VS solubilisation and methane yield increase (R^2 = 0.95) (Fig. 2a), i.e. the higher the organic matter solubilisation, the higher methane yield increase. Similarly, a positive correlation was found between carbohydrate solubilisation and methane yield increase (R^2 = 0.96) (Fig. 2b). Carbohydrates are the main macromolecules composing microalgae cell wall in *Monoraphidium* sp. and *Stigeoclonium* sp., which suggests that pretreatments enhanced the anaerobic bioavailability and biodegradability of the cell wall compounds. Regarding proteins, the most abundant macromolecule in microalgal biomass, a positive correlation was found between protein solubilisation vs. methane yield increase considering all pretreatments but the hydrothermal one, which actually showed the highest lipid solubilisation. In fact, no correlation was found between FAME solubilisation and methane yield increase, which was the macromolecule with the lowest solubilisation after all pretreatments except for hydrothermal.

Comparing the pretreatments methods assayed, thermal pretreatment may have reached the highest methane yield due to a longer exposure time (10 hours) in comparison with hydrothermal pretreatment (15 min). However, in other studies thermal pretreatment at high temperature and pressure (170 ºC and 6 bars) for 15 min, known as steam explosion, was the best technique (Alzate et al., 2012).

Microwave and ultrasound pretreatments may have reached better results if higher specific energies were applied. For instance, when *Scenedesmus* biomass was sonicated at 35-76 MJ/kg TS there was no or little increase in methane yield (0-14%), while this increase was significant at 100-130 MJ/kg TS (75-88%) (González-Fernández et al., 2012). This was also observed for other organic substrates. Hydrothermal pretreatment at 200ºC attained the highest sugar solubilisation and methane yield increase for sugarcane bagasse compared to acid and alkaline pretreatments. In fact, temperature was found to be the most influencing independent variable. The higher the temperature, the higher the anaerobic digestion performance (Costa et al., 2014). Similarly, pulp and paper mill wastewater was pretreated using 12 different methods and the best one was hydrothermal pretreatment at 150 ºC, which caused the disruption of chemical bonds in cell walls and membranes (Bayr et al., 2013). Nonetheless, more severe pretreatment conditions require higher energy input.

The energy balance of pretreatments is crucial for full-scale implementation. Indeed, the heat and electricity demand for pretreatment and anaerobic digestion must be lower than the extra energy generated from biogas. Our previous studies evaluated the energy balance of microwave, thermal and hydrothermal pretreatments and the anaerobic digestion process by extrapolating experimental data from laboratory-scale continuous reactors to full-scale systems. The results showed how mechanical pretreatments, like microwave, required a high electricity input which unbalanced the process. Low biomass concentration was among the main factor determining the high energy input of these processes, requiring biomass dewatering (15-20% TS) (Passos et al., 2014c). On the other hand, hydrothermal pretreatment achieved neutral energy balance after biomass thickening (5-10% TS). Finally, thermal pretreatment attained a positive energy balance (i.e. 20% more energy produced than consumed) even without biomass thickening (< 5% TS) (Passos and Ferrer, 2014).

To sum up, thermal pretreatment not only enhanced microalgal biomass solubilisation and methane yield, but it may also lead to net energy production.

4. Conclusions

Thermal, hydrothermal, microwave and ultrasound pretreatments were compared in terms of microalgae solubilisation and methane yield increase in BMP tests. Organic matter solubilisation increase was much higher for thermal pretreatment (20-fold) when compared to hydrothermal (9-fold), microwave (8-fold) and ultrasound (7-fold) pretreatments. For thermal, microwave and ultrasound pretreatments, proteins and carbohydrates were the main macromolecules solubilised, which was attributed to the macromolecular composition of microalgal biomass and cell wall. The methane yield was significantly higher after thermal pretreatment (72% increase), followed by hydrothermal (28%) and microwave (21%) pretreatments, while ultrasound was not improved as compared to non-pretreated biomass. A positive correlation was found between volatile solid solubilisation and methane yield increase, as well as between carbohydrate solubilisation and methane yield increase. Overall, the best results were obtained with thermal pretreatment (95 ºC, 10 hours), which ought to be evaluated at pilot scale.

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Table 1. Best pretreatment conditions for improving the anaerobic digestion of microalgal biomass grown in wastewater treatment algal ponds.

Pretreatment	Applied conditions	Best condition	Solubilisation increase (%)	Methane yield	Reference
Thermal	Temperature (55, 75, 95 ºC); Exposure time (5, 10, 15 h)	95 ºC; 10 h	20.6	170 mL CH_4/g VS (61% increase)	Passos et al., 2013a
Hydrothermal	Temperature (110, 130 ºC); Exposure time (15, 30 min)	130 ºC; 15 min	15.0	169 mL CH_4/g VS (39% increase)	Passos and Ferrer, 2014
Microwave	Output power (300, 600, 900 W); Exposure time (1-9 min); Specific energy (16-67 MJ/kg TS)	900 W; 3 min	7.6	209 mL CH_4/g VS (78% increase)	Passos et al., 2013b
Ultrasound	Output power (50, 60, 70 W); Exposure time (10, 20, 30 min); Specific energy (21-65 MJ/kg TS)	70 W; 30 min	91	196 mL CH_4/g COD (33% increase)	Passos et al., 2014b

Table 2. Microalgal biomass and inoculum characteristics. Mean values (standard deviation).

Parameter	**Microalgal biomass**	**Inoculum**
pH	7.23 (0.15)	7.36 (0.06)
TS (g/L)	31.49 (0.41)	33.24 (0.17)
VS (g/L)	20.19 (0.24)	22.76 (0.06)
VS/TS (%)	64.1 (0.32)	68.5 (0.15)
COD (g/L)	28.8 (0.40)	31.3 (0.26)
Proteins (%)	58 (4)	-
Carbohydrates (%)	22 (3)	-
Lipids (%)	19 (3)	-

Table 3. Microalgal biomass solubilisation under different pretreatment conditions.

Pretreatment	Applied conditions	Soluble organic matter		Soluble proteins		Soluble carbohydrates		Soluble lipids	
		Concentration (g VSs/L)	Increase	Concentration (mg proteins/L)	Increase	Concentration (mg carbohydrates/L)	Increase	Concentration (mg FAME/L)	Increase
Control	-	0.29[a]	-	11 (2.1)[a]	-	79 (5.8)[a]	-	3 (1.3)[a]	-
Thermal	95 ºC; 10 h	5.73[d]	20-fold	563 (5.2)[d]	51-fold	2349 (8.6)[d]	30-fold	39 (4.4)[c]	13-fold
Hydrothermal	130 ºC; 15 min	2.64[c]	9-fold	254 (4.3)[c]	23-fold	879 (5.3)[c]	11-fold	93 (2.8)[d]	31-fold
Microwave	900 W; 3 min (34.3 MJ/kg TS)	2.23[b]	8-fold	193 (3.8)[c]	18-fold	915 (8.2)[c]	12-fold	5 (0.9)[a]	2-fold
Ultrasound	70 W; 30 min (26.7 MJ/kg TS)	2.17[b]	7-fold	135 (4.2)[b]	12-fold	690 (4.3)[b]	9-fold	9 (2.1)[b]	3-fold

Note: [a,b,c,d] Stand for significant differences in columns with concentration values ($\alpha = 5\%$)

Table 4. Anaerobic digestion of microalgal biomass under different pretreatment conditions.

Pretreatment	Anaerobic digestion rate (d^{-1})	Methane yield (mL CH_4/g VS)
Control	0.110 (0.003) [a]	105.6 (2.2) [a]
Thermal	0.123 (0.010) [a]	181.3 (5.5) [c]
Hydrothermal	0.114 (0.011) [a]	134.9 (2.0) [b]
Microwave	0.122 (0.009) [a]	127.7 (4.7) [b]
Ultrasound	0.130 (0.007) [a]	113.7 (2.1) [a]

Note: [a,b,c] Stand for significant differences in each column ($\alpha = 5\%$)

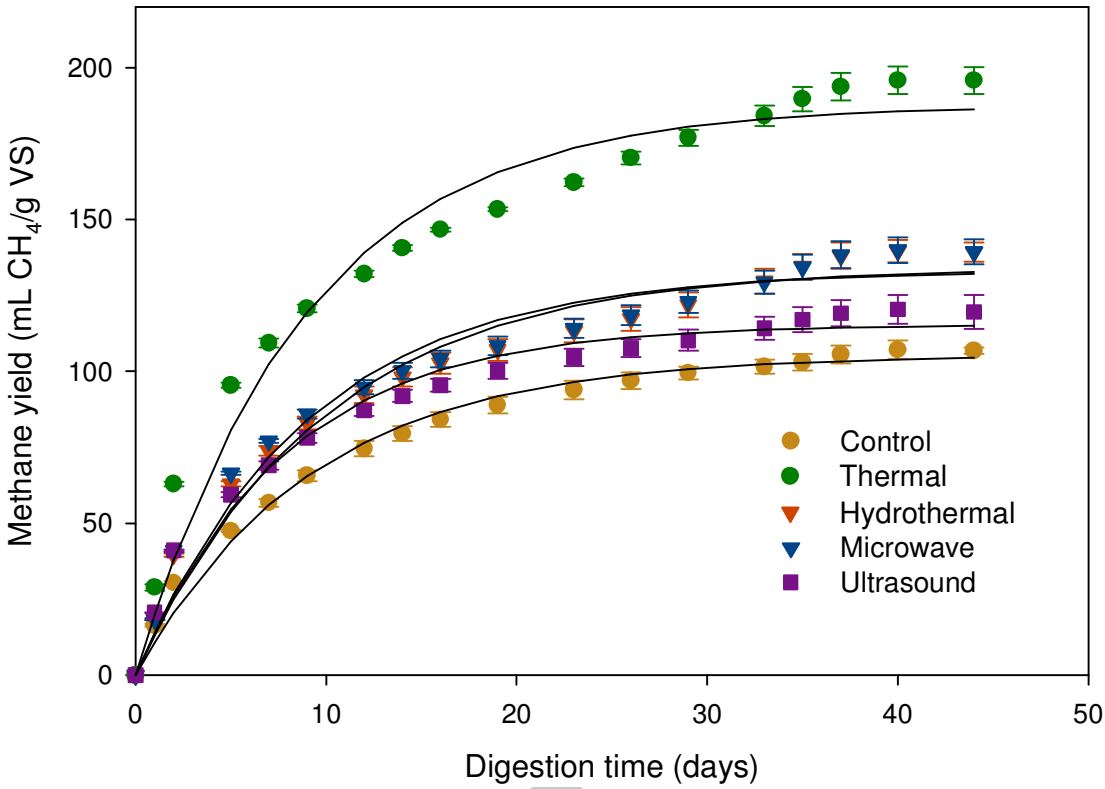
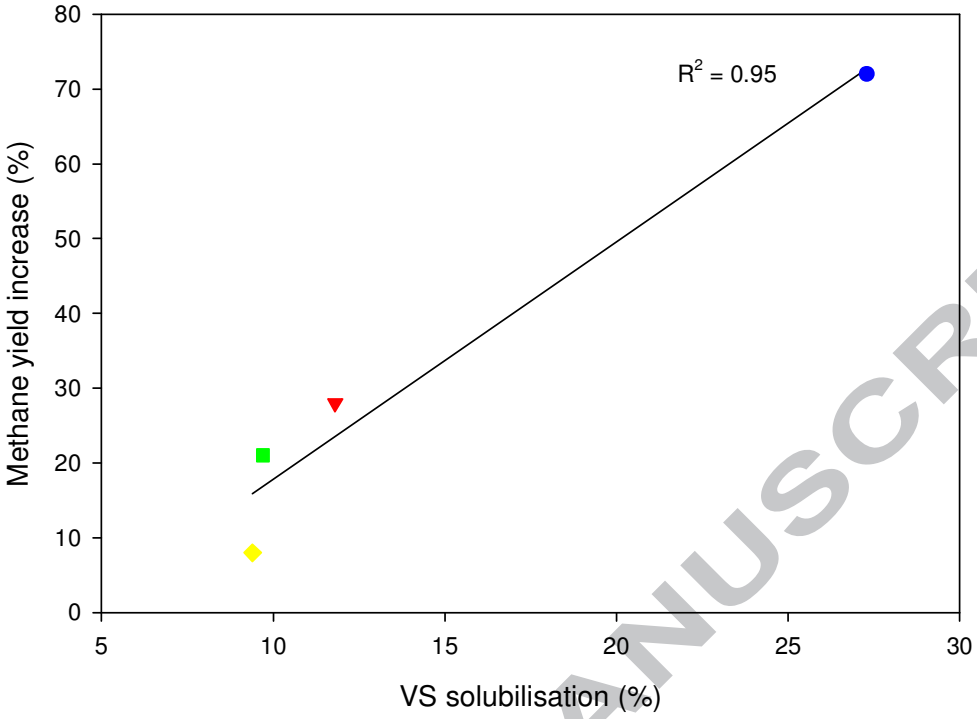
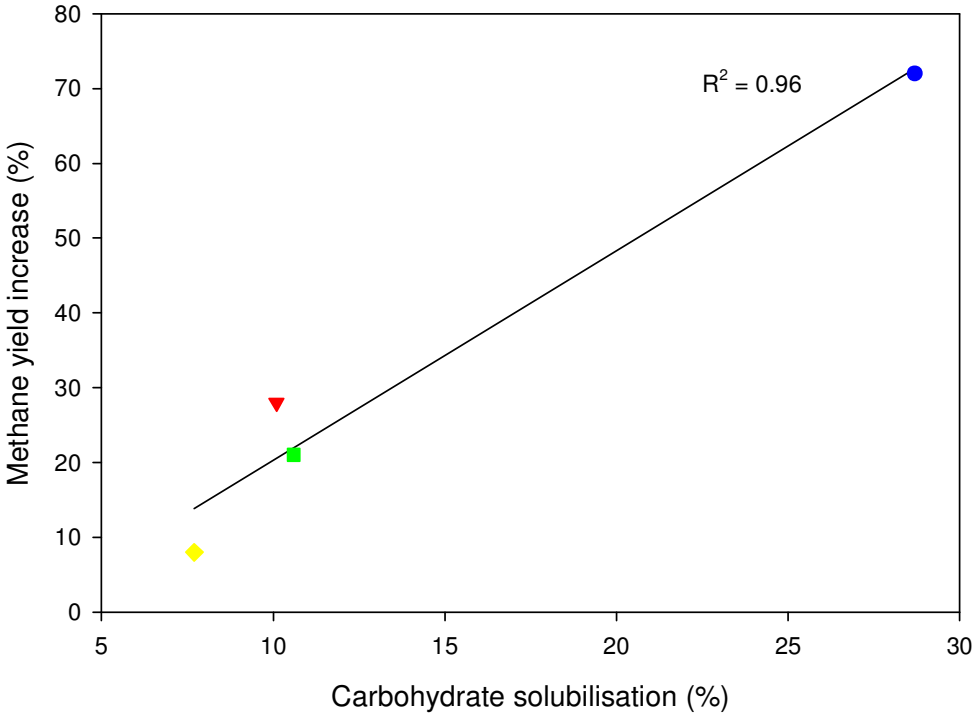


Figure 1. Microalgal biomass methane yield after thermal and mechanical pretreatments.



(a)



(b)

Figure 2. Correlation between microalgal biomass solubilisation and methane yield increase after thermal and mechanical pretreatment methods, where (a) shows volatile solids (VS) solubilisation vs. methane yield increase and (b) carbohydrates solubilisation vs. methane yield increase. Note that the blue circle represents thermal pretreatment, the red triangle represents hydrothermal pretreatment, the green square represents microwave pretreatment and the yellow diamond represents ultrasound pretreatment.

Highlights

1. The effect of thermal and mechanical pretreatments on microalgal biomass was compared
2. The highest biomass solubilisation was attained for thermal pretreatment (95ºC, 10 h)
3. The highest methane yield increase (72%) was also attained for thermal pretreatment
4. Biomass solubilisation and methane yield increase showed a positive correlation